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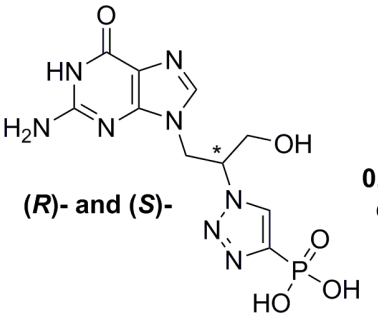


**(*R*)- and (*S*)-**

**1-6 µM** inhibition of plasmodial HG(X)PRT

**0.1-0.4 µM** inhibition of human HGPRT

# Novel nucleotide analogues bearing (1*H*-1,2,3-triazol-4-yl)phosphonic acid moiety as inhibitors of *Plasmodium* and human 6-oxopurine phosphoribosyltransferases

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## Abstract

A novel family of acyclic nucleoside phosphonates (ANPs) bearing a (1*H*-1,2,3-triazol-4-yl)phosphonic acid group in the acyclic side chain have been prepared in order to study the influence of the hetaryl rigidizing element on the biological properties of such compounds. The key synthetic step consisted of a copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) between diethyl ethynylphosphonate and the corresponding azidoalkyl precursor. Two ANPs in this family, bearing a guanine base, exhibited the highest potency for the human 6-oxopurine phosphoribosyltransferase irrespective of the stereochemistry on the C-2' atom. Four compounds inhibited *Plasmodium falciparum* 6-oxopurine phosphoribosyltransferase with little differences in their $K_i$ values irrespective of whether the base was guanine, hypoxanthine or xanthine but only two, with guanine as base, inhibited *Pv*HGPRT.

**Keywords:** acyclic nucleoside phosphonates; 6-oxopurine; hypoxanthine-guanine-(xanthine) phosphoribosyltransferase, copper(I)-catalyzed azide-alkyne cycloaddition

## Introduction

Acyclic nucleoside phosphonates (ANPs)[1] represent an important class of antimetabolites that mimic the naturally occurring nucleoside monophosphates. Extensive structure-activity relationship (SAR) studies have been carried out and several distinct classes of ANPs with diverse biological activities have been identified. 2-(Phosphonomethoxy)ethyl or PME (*e.g.* PMEA, Fig. 1), 2-(phosphonomethoxy)propyl or PMP, and 3-hydroxy-2-

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(phosphonomethoxy)propyl or HPMP (*e.g.* (*S*)-HPMPC, Fig. 1) analogues have antiviral properties,[2-4] and 2-(phosphonoethoxy)ethyl or PEE derivatives (such as PEEHx and PEEG, Fig. 1), bisphosphonates (Fig. 1) and aza-ANPs (Fig. 1) have antimalarial[5-9] and/or antimycobacterial[10-11] activity. Several different chemical types of ANPs, including modified PMEA analogues, have also been studied as potent inhibitors of bacterial adenylate cyclases, namely adenylate cyclase toxin from *Bordetella pertussis* and edema factor from *Bacillus anthracis*.[12-14] Such analogues may have potential for treatment or prevention of toxaemia caused by the invasion of these bacteria into the human host.

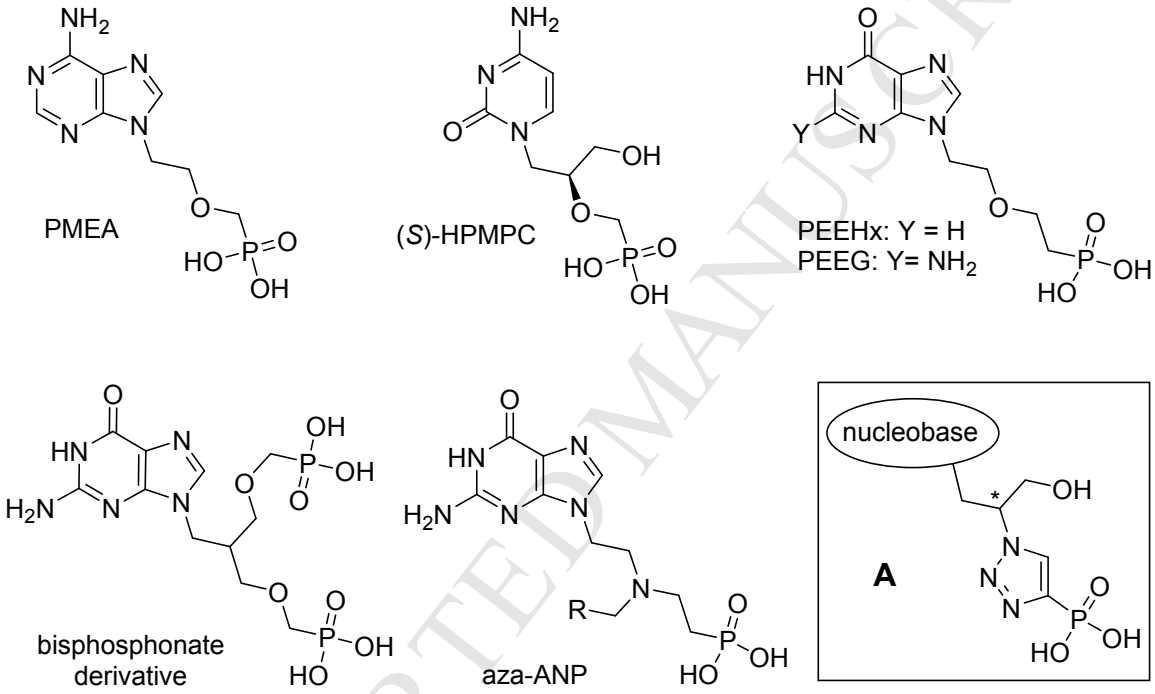



**Figure 1.** Examples of chemical structures of biologically active acyclic nucleoside phosphonates (ANPs). Top row: The antiviral compounds PMEA and (*S*)-HPMPC, and antimalarial compounds PEEHx and PEEG. Bottom row: an antiplasmodial bisphosphonate, the general structure of the aza-ANPs, and the general scaffold of the newly designed (1*H*-1,2,3-triazol-4-yl)phosphonates **A**.

Inhibition of plasmodial hypoxanthine-guanine-(xanthine) phosphoribosyltransferases (HG(X)PRTs) by the ANPs is well-correlated with their antimalarial activity.[5-9] These enzymes catalyze the formation of the 6-oxopurine mononucleotides from the 6-oxopurine nucleobases and 5-phospho-$\alpha$-D-ribosyl-1-pyrophosphate (Fig. 2).[15] HG(X)PRTs are key enzymes of the purine salvage pathway and since malarial parasites lack the *de novo* pathway

for purine nucleotide synthesis, this enzyme is a validated target for the development of new antimalarials.[5-9] Importantly, the mode of action of the ANPs is different from the currently used drugs, so represents a new approach to developing antimalarial therapeutics.

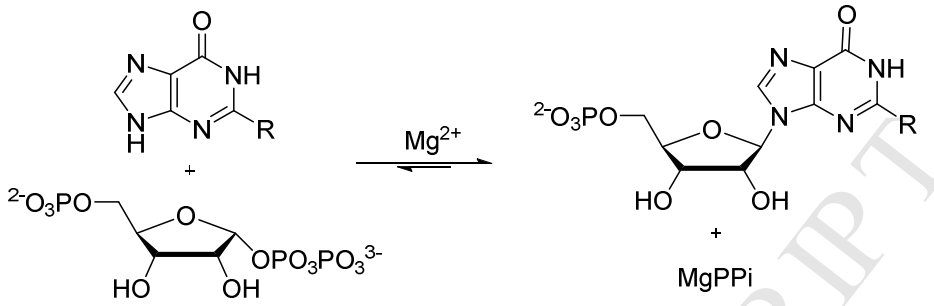



**Figure 2**. Reaction catalyzed by the HG(X)PRTs. The naturally occurring bases are hypoxanthine (R = H), guanine (R = $NH_2$) and xanthine (R = OH).

Novel types of ANPs bearing (1*H*-1,2,3-triazol-4-yl)phosphonic acid group attached to the acyclic side chain (general structure **A**, Fig. 1) have been synthesized as a continuation of the extended SAR studies carried out by our group. Compounds **A** (Fig. 1) bearing 6-oxopurine bases were designed as potential inhibitors of plasmodial HG(X)PRTs since it has been reported[16] that the optimal length of the aliphatic linker between the nucleobase and the phosphonate group is 5 or 6 atoms (in contrast to antiviral ANPs with 4-atom-linkers). In comparison to the flexible PEE or modified PEE analogues which are potent HG(X)PRTs inhibitors, derivatives **A** (Fig. 1) have the 1*H*-1,2,3-triazol-4-yl moiety integrated into the acyclic chain to rigidify the linker, thus, possibly leading to increased affinity.

An efficient synthetic methodology to access the desired 6-oxopurine ANPs with the (1*H*-1,2,3-triazol-4-yl)phosphonic acid moiety has been developed and optimized. To show the full scope of this synthetic approach, the whole series of ANPs with various purine or pyrimidine bases attached were prepared in good overall yields.

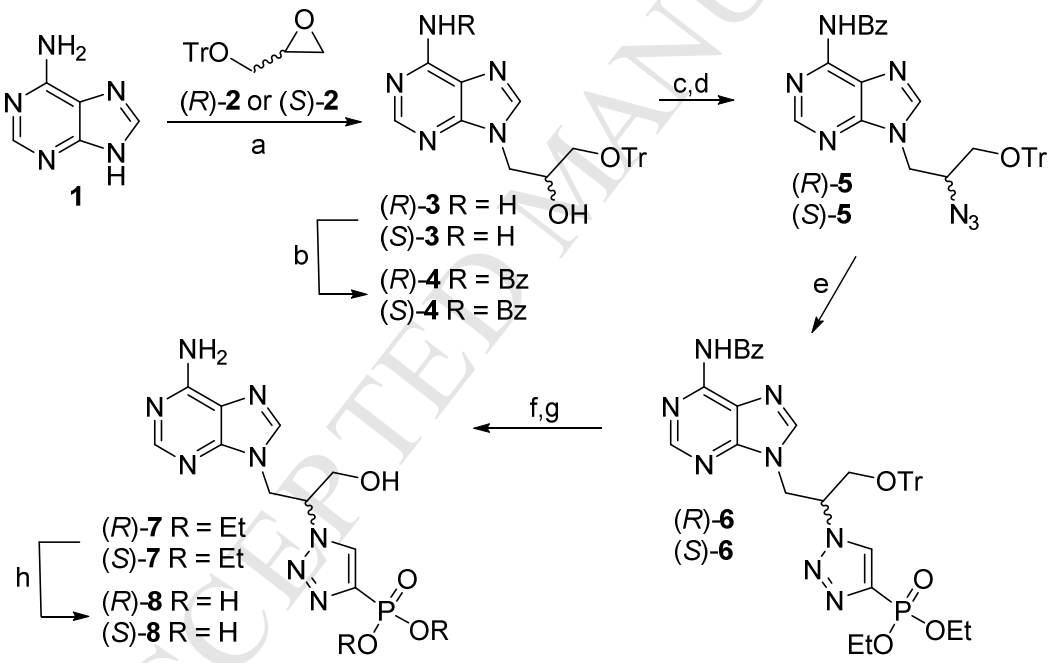
## Results and Discussion

### *Chemistry.*

Since the designed compounds **A** (Fig. 1) contain a stereogenic centre at the C-2' atom, both (*R*)- and (*S*)-enantiomers were synthesized side by side for their subsequent biological evaluations. The key synthetic step involved the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) between diethyl ethynylphosphonate[17] and suitable intermediate bearing an azido group. In general, two strategies could be utilized for the introduction of the

1,2,3-triazole ring into target ANPs: a) using the "click" CuAAC chemistry between diethyl ethynylphosphonate and acyclic nucleoside analogue having the azido group in the side aliphatic chain or b) the preparation of suitable intermediate bearing diethyl (1*H*-1,2,3-triazol-4-yl)phosphonate moiety for its subsequent attachment to purine or pyrimidine nucleobases.

The first approach was applied for the synthesis of adenine ANPs by analogy to previously reported procedures.[18,19] The synthesis started with alkylation of adenine with commercially available enantiomerically pure tritylated (*R*)-(+)- and (*S*)-(–)-glycidols, (*R*)-**2** and (*S*)-**2** (Scheme 1), to give compounds (*R*)-**3** and (*S*)-**3**, respectively.[18] Intermediates (*R*)-**3** and (*S*)-**3** were then benzoylated at the exocyclic amino group to form derivatives (*R*)-**4** and (*S*)-**4**,[18] which were further converted into their mesylate derivetives and, subsequently, to azido derivatives (*R*)-**5** and (*S*)-**5**, respectively, using $NaN_3$ (Scheme 1).[19]

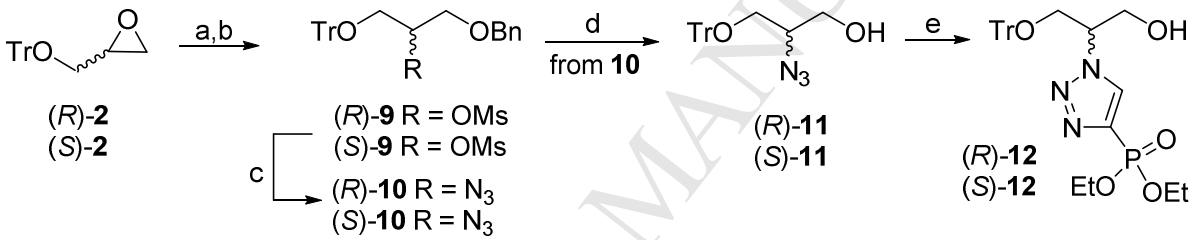



**Scheme 1.** Reagents and conditions: a) NaH, DMF, 105 °C, 16 h; b) $Me_3SiCl$, Py, rt, 2h and then PhCOCl, Py, rt, 2 h; c) MsCl, Py, rt, 4 h; d) $NaN_3$, DMF/HMPA, 100 °C, 12 h; e) $HC{\equiv}C(P)(O)(OEt)_2$, CuI, DiPEA, DMF; f) $MeNH_2$ in MeOH (8M solution), toluene, rt, 4 h; g) 80% aq. $CH_3COOH$, 90 °C, 4 h; h) $Me_3SiBr$, MeCN, rt, 24 h, then aq. MeOH.

The CuAAC cycloaddition between azido compounds (*R*)-**5** or (*S*)-**5** and diethyl ethynylphosphonate,[17] using CuI and DiPEA in DMF,[20] provided the desired 1,4-substituted triazole derivatives (*R*)-**6** or (*S*)-**6** in good yields (Scheme 1). The removal of benzoyl and

trityl groups using methylamine in toluene[21] and 80% aq. acetic acid, respectively, followed by removal of phosphonate ethyl ester moieties with $Me_3SiBr$/MeCN with ensuing hydrolysis,[22] afforded final products (*R*)-**8** or (*S*)-**8** (Scheme 1).

The second synthetic strategy seems to be more efficient and more broadly applicable since the preformed aliphatic precursor bearing (1*H*-1,2,3-triazol-4-yl)phosphonate group can be directly attached to suitably modified purines or pyrimidines. At first, the starting tritylated (*R*)-(+)- and (*S*)-(–)-glycidols, compounds (*R*)-**2** and (*S*)-**2** (Scheme 2), were successively treated with freshly prepared sodium benzyloxide in DMF (without isolation of the products),[23] with MsCl in pyridine (to give (*R*)-**9** and (*S*)-**9**, respectively), and finally with $NaN_3$ in a DMF/HMPA mixture to afford azido derivatives (*R*)-**10** and (*S*)-**10**, respectively, in overall yields higher than 60% (Scheme 2).

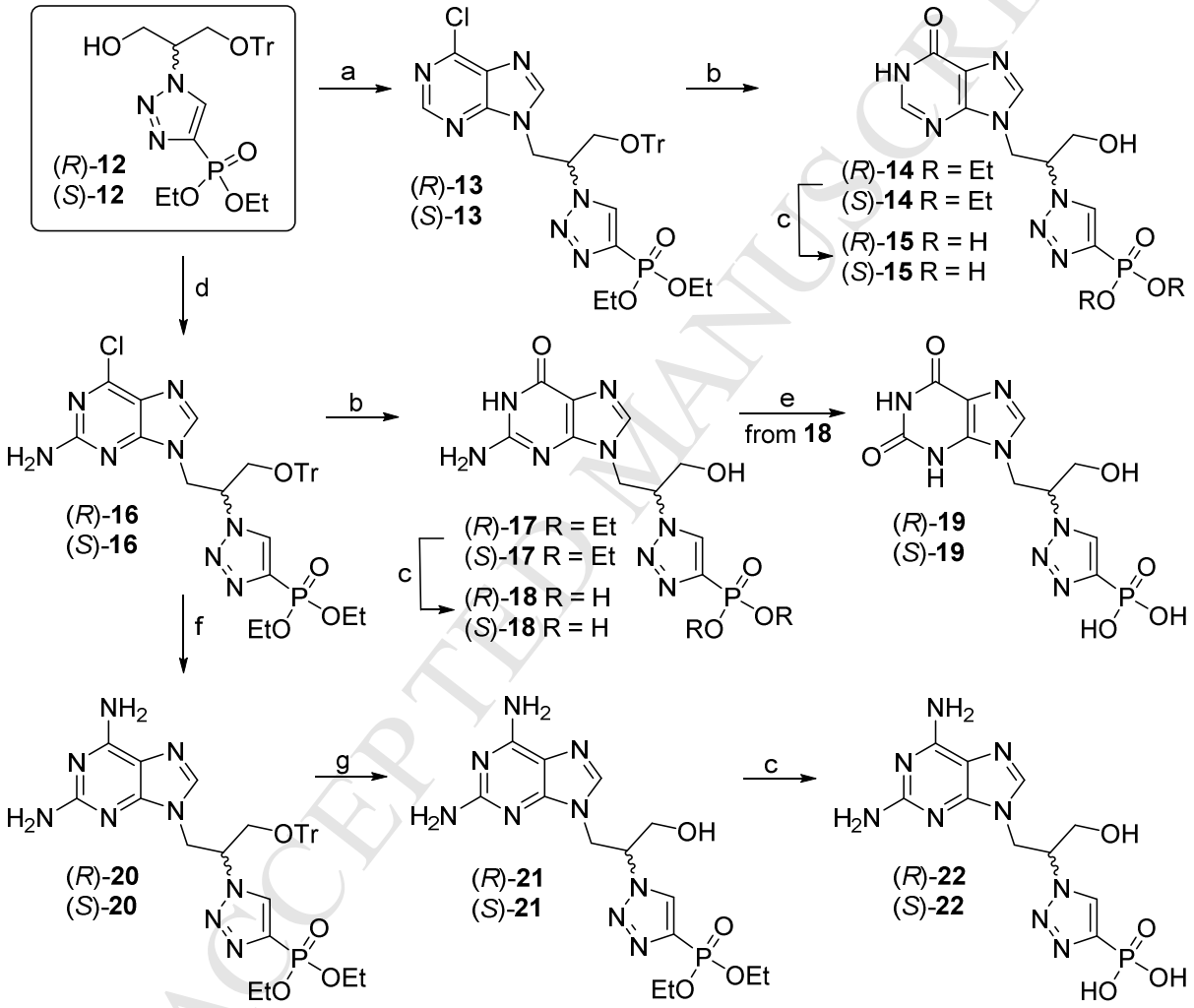



**Scheme 2.** Reagents and conditions: a) NaH, DMF, $PhCH_2OH$, 100 °C, 2 h; b) MsCl, Py, rt, 4 h c) $NaN_3$, DMF/HMPA; 100 °C, 5 h; d) $NaBrO_3$, $Na_2S_2O_4$, EtOAc, $H_2O$; e) $HC{\equiv}C(P)(O)(OEt)_2$, CuI, DiPEA, DMF.

To prepare the desired 1,2,3-triazole intermediates (*R*)-**12** and (*S*)-**12** (Scheme 2), azides (*R*)-**10** and (*S*)-**10** can be either first debenzylated and then cyclized under the CuAAC cycloaddition conditions or first cyclized and then debenzylated. Both approaches were tentatively tested and the former approach was selected as it gave higher yields. Thus, removal of the benzyl group from compounds (*R*)-**10** and (*S*)-**10**, using a $NaBrO_3/Na_2S_2O_4$ reagent under two-phase conditions (a method compatible with the present azido group),[24] afforded hydroxyl derivatives (*R*)-**11** and (*S*)-**11**, respectively, which were then converted by the above described CuAAC cycloaddition[20] with diethyl ethynylphosphonate[17] to the desired precursors (*R*)-**12** and (*S*)-**12** in satisfactory overall yields (Scheme 2).

The next crucial synthetic step consisted of the attachment of 1,2,3-triazole intermediates (*R*)-**12** and (*S*)-**12** to appropriate purine or pyrimidine nucleobases *via* Mitsunobu reaction. First,

6-chloropurine and 2-amino-6-chloropurine were reacted with compounds (*R*)-**12** and (*S*)-**12** in the presence of $PPh_3$ and DIAD in dioxane at room temperature to give 6-chloropurine derivatives (*R*)-**13** and (*S*)-**13**, and 2-amino-6-chloropurine analogues (*R*)-**16** and (*S*)-**16** in good yields (Scheme 3). The final free phosphonates (*R*)-**15**, (*S*)-**15**, (*R*)-**18** and (*S*)-**18** were obtained by simultaneous hydrolysis of the 6-chloropurine group and detritylation using 75% aq. $CF_3COOH$ at room temperature (to give 6-oxopurine intermediates **14** and **17**), followed by the standard removal of the phosphonate ethyl ester moieties (Scheme 3).



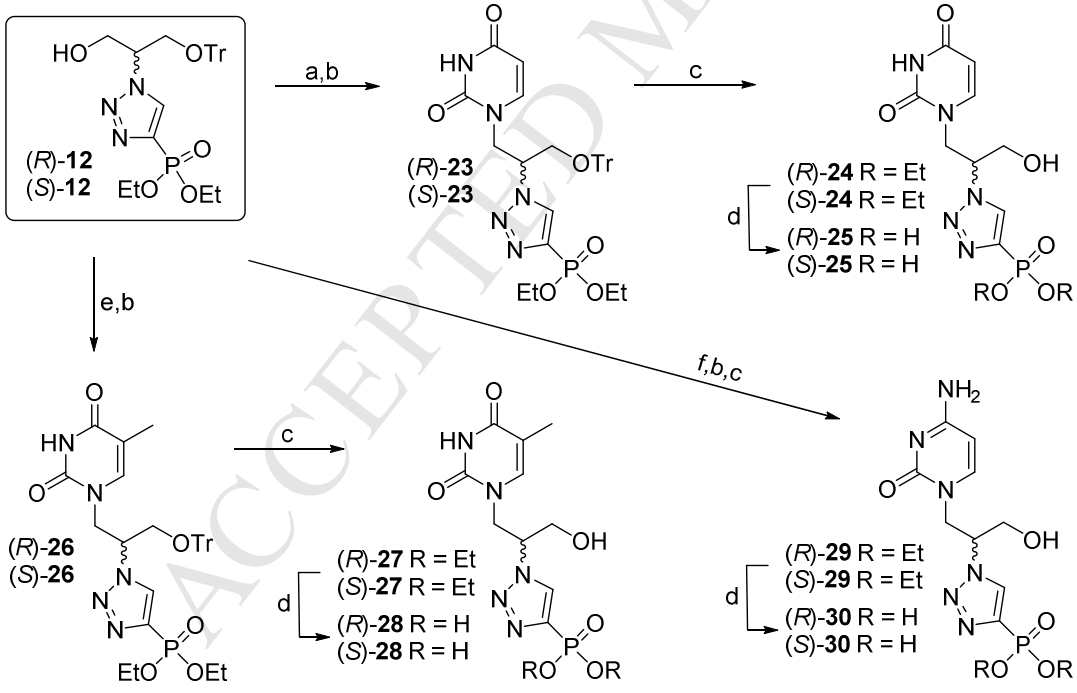

**Scheme 3.** Reagents and conditions: a) 6-chloropurine, $PPh_3$, DIAD, dioxane, rt, 48 h; b) 75% aq. $CF_3COOH$, rt, 48 h; c) TMSBr, MeCN, rt, 24 h, then aq. MeOH; d) 2-amino-6-chloropurine, $PPh_3$, DIAD, dioxane, rt, 48 h; e) isoamyl nitrite, 80% aq. $CH_3COOH$, rt, 12 h; f) $NH_3$, EtOH, 100 °C, 24 h; g) 80% aq. $CH_3COOH$, 90 °C, 2 h.

The amino group at C-2 position of compounds (*R*)-**18** and (*S*)-**18** was replaced with an oxo group using standard diazotization-hydroxydediazoniation approach (treatment with isoamyl

nitrite in 80% acetic acid) to afford the corresponding xanthine derivatives (*R*)-**19** and (*S*)-**19**, respectively, in 45% yields (Scheme 3).

Ammonolysis of 2-amino-6-chloropurine intermediates (*R*)-**16** and (*S*)-**16** gave 2,6-diaminopurine derivatives (*R*)-**20** and (*S*)-**20** which, after detritylation (to yield **21**) and subsequent removal of the ethyl ester groups, afforded the final phosphonates (*R*)-**22** and (*S*)-**22** (Scheme 3).

Next, the corresponding ANPs containing pyrimidine nucleobases were synthesized. The Mitsunobu procedure[25] (compounds (*R*)-**12** or (*S*)-**12**, $PPh_3$, DIAD, dioxane, room temperature), followed by nucleobase *N*-debenzoylation with propylamine in dioxane, was employed for alkylation of 3-benzoyluracil[25] and 3-benzoylthymine[25] to form pyrimidine intermediates (*R*)-**23**, (*S*)-**23** and (*R*)-**26**, (*S*)-**26**, respectively (Scheme 4).[26] Detritylation of compounds **23** and **26** (to yield hydroxy derivatives **24** and **27**) and subsequent removal of the ethyl ester groups gave final phosphonates (*R*)-**25**, (*S*)-**25** and (*R*)-**28**, (*S*)-**28**, respectively (Scheme 4).




**Scheme 4.** Reagents and conditions: a) $N^3$-benzoyluracil, $PPh_3$, DIAD, dioxane, rt, 48 h; b) propylamine, dioxane, rt, 12 h; c) 80% aq. $CH_3COOH$, 90 °C, 2 h; d) TMSBr, MeCN, rt, 24 h, then aq. MeOH; e) $N^3$-benzoylthymine, $PPh_3$, DIAD, dioxane, rt, 48 h; f) $N^4$-benzoylcytosine, $PPh_3$, DIAD, dioxane, rt, 48 h.

Analogously, $N^4$-benzoylcytosine[27] was treated with (*R*)-**12** or (*S*)-**12** under reported Mitsunobu reaction conditions,[25] followed by a subsequent removal of the benzoyl and trityl groups to give cytosine derivatives (*R*)-**29** and (*S*)-**29**, respectively (Scheme 4). Standard removal of the ethyl ester groups from (*R*)-**29** and (*S*)-**29** produced the final compounds (*R*)-**30** and (*S*)-**30**, respectively (Scheme 4).

***Biological activity***

Considering the important biological properties of ANPs in general, free phosphonates (*R*)-**8**, (*S*)-**8**, (*R*)-**15**, (*S*)-**15**, (*R*)-**18**, (*S*)-**18**, (*R*)-**19**, (*S*)-**19**, (*R*)-**22**, (*S*)-**22**, (*R*)-**25**, (*S*)-**25**, (*R*)-**28**, (*S*)-**28**, (*R*)-**30** and (*S*)-**30** were tested in standard antiviral assays,[28] but no significant antiviral activity was observed against viruses tested, *i.e.* against human immunodeficiency virus 1 (HIV-1), human rhinovirus (HRV) and hepatitis C virus (HCV).

ANPs bearing 6-oxopurine bases, namely analogues with hypoxanthine (compounds **15**), guanine (compounds **18**) or xanthine (compounds **19**) as the purine base have been designed as potential inhibitors of hypoxanthine-guanine-(xanthine) phosphoribosyltransferases [HG(X)PRTs].[5-9] Thus, the compounds were tested as potential inhibitors of human HGPRT, *Plasmodium falciparum* HGXPRT and *Plasmodium vivax* HGPRT. The hypoxanthine derivatives (*R*)-**15** and (*S*)-**15** showed an inhibition of the human HGPRT and *Pf*HGXPRT (only isomer (*R*)-**15**) with $K_i$ values as low as 3 μM range (Table 1). These compounds did not inhibit the *Pv* enzyme. The most potent ANPs in this series are the guanine analogues (*R*)-**18** and (*S*)-**18** which are submicromolar inhibitors of human HGPRT and low micromolar inhibitors of both *Pf*HGXPRT and *Pv*HGPRT (Table 1). Interestingly, the potent inhibitory properties of compounds **18** did not depend distinctly on the stereochemistry on the C-2' atom, as both (*R*)-**18** and (*S*)-**18** isomers exhibited similar $K_i$ values against all three HG(X)PRTs tested (Table 1). On the other hand, in the case of xanthine analogues only isomer (*R*)-**19** (similarly as in the hypoxanthine series) was a potent inhibitor and selective for *Pf*HGXPRT (Table 1). This is because xanthine is not a substrate for the human or *Pv* enzymes and compounds containing this base cannot, in consequence, bind in the active site. However, if the orientation of the compound varies so that the location of the base is not identical to that of with hypoxanthine or guanine, there remains a possibility that they could bind to these two enzymes.

**Table 1.** $K_i$ values of the ANPs bearing three different 6-oxopurine nucleobases for human HGPRT, *Pf*HGXPRT and *Pv*HGPRT.

| Compound | nucleobase | $K_i$ [μM] | | |
|---|---|---|---|---|
| | | human | *Pf* | *Pv* |
| (*R*)-**15** | hypoxanthine | **8** | **3** | >100 |
| (*S*)-**15** | hypoxanthine | **7** | >50 | >100 |
| (*R*)-**18** | guanine | **0.1** | **1** | **6** |
| (*S*)-**18** | guanine | **0.4** | **2** | **2** |
| (*R*)-**19** | xanthine | >500 | **2** | >200 |
| (*S*)-**19** | xanthine | >500 | >50 | >500 |

## Conclusions

A methodology for the synthesis of acyclic nucleoside phosphonates (ANPs) bearing (1*H*-1,2,3-triazol-4-yl)phosphonate moiety in the acyclic side chain has been developed and a series of 16 compounds in both (*R*)- and (*S*)-conformations was prepared for their biological evaluation. Six of these compounds were investigated as inhibitors of the human, *Pf* and *Pv* HG(X)PRTs. Two of these ANPs with guanine as the purine base were found to be submicromolar inhibitors of human HGPRT, *i.e.* with lower $K_i$ values than when hypoxanthine is the base. This is consistent with the fact that the $K_m$ for guanine for this enzyme is lower than for hypoxanthine. The guanine analogues also inhibited the two *plasmodial* enzymes but with higher $K_i$ values. Guanine bearing ANPs were the most potent HG(X)PRTs inhibitors, irrespectively of the stereochemistry on the C-2' atom of the aliphatic linker, but they lack selectivity for the plasmodial enzymes. The xanthine compound (*R*)-**19** with the $K_i$ value of 2 μM against *Pf*HGXPRT is analogue with best selectivity (no inhibition of human HGPRT) and, thus, represents the most promising compound for the future synthesis of suitable prodrugs and their further biological evaluations as potential antimalarial agents.

## Experimental section

Starting compounds and reagents were purchase from commercial suppliers or were prepared according to the published procedures. Solvents were dried by standard procedures. Solvents were evaporated at 40 °C/2 kPa. Analytical TLC was performed on plates of Kieselgel 60 $F_{254}$ from Merck. NMR spectra were recorded on Bruker Avance 400 spectrometer ($^1$H at 400 MHz, $^{13}$C at 100.6 MHz, $^{31}$P at 161.9 MHz) with TMS or dioxane (3.75 ppm for $^1$H, 67.19

ppm for $^{13}C$ NMR) as internal standard or referenced to the residual solvent signal. Mass spectra were measured on UPLC-MS (Waters SQD-2). HR MS were taken on a LTQ Orbitrap XL spectrometer. The purity of the tested compounds was determined by HPLC ($H_2O$-$CH_3CN$, linear gradient) and was higher than 95%.

**General procedure 1 (GP1). Copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC).**[20] Diisopropylethylamine (1.7 ml, 10 mmol) and CuI (190 mg, 1 mmol) were added to the mixture of starting azido derivative (5 mmol) and diethyl ethynylphosphonate[17] (970 mg, 6 mmol) in anhydrous DMF (30 mL) under argon at room temperature. The reaction mixture was stirred at room temperature for 4 h and then diluted with EtOAc (50 mL). Organic phase was washed with water (3 × 50 mL) water, dried over anhydrous $MgSO_4$, filtered, concentrated *in vacuo*, and purified by chromatography over silica gel ($CHCl_3$ → $CHCl_3$/MeOH, 100/3, v/v) to give the corresponding (1*H*-1,2,3-triazol-4-yl)phosphonic acid derivative.

**General procedure 2 (GP2). Synthesis of free phosphonic acids.**[22] A mixture of the corresponding diethyl ester phosphonate (1.0 mmol), acetonitrile (5 mL), and TMSBr (1.0 mL) was stirred at room temperature for 24 h. After evaporation and codistillation with acetonitrile (2 × 5 mL), the residue was treated with aqueous methanol (2:1, 10 mL) for 30 min, evaporated to dryness and crystallized/precipitated to afford the final phosphonic acids as crystals/solid.

**General procedure 3 (GP3). Mitsunobu reaction.**[25] Diisopropylazadicarboxylate (DIAD, 14 mmol) was added dropwise to the solution of triphenylphosphine (15 mmol), alcohol (*R*)-**12** or (*S*)-**12** (5 mmol), and the corresponding heterocyclic base (7.5 mmol) in dry dioxane or THF (100 mL). The reaction mixture was stirred at room temperature for 48 h, evaporated *in vacuo*, and purified by column chromatography over silica gel.

**General procedure 4 (GP4). Detritylation.** A mixture of a trityl derivative (1.0 mmol) in 80% aq. acetic acid (2.5 mL) was heated at 90 °C for 2 h. Volatiles were evaporated and the crude product was purified by chromatography over silica gel.

**(*R*)-1-(6-Amino-*9H*-purin-9-yl)-3-(trityloxy)propan-2-ol (*R*)-3 and (*S*)-1-(6-Amino-*9H*-purin-9-yl)-3-(trityloxy)propan-2-ol (*S*)-3.** In analogy to the reported procedure,[18] a mixture

of adenine (**1**, 4.73 g, 35 mmol) and NaH (60% in mineral oil, 280 mg, 7 mmol) in DMF (75 mL) was stirred at room temperature for 2 h. Tritylated glycidol (*R*)-**2** or (*S*)-**2** (9.5 g, 30 mmol) in DMF (100 mL) was added, and the resulting solution was heated at 105 °C for 16 h. Solvent was evaporated, the residue was extracted with EtOAc (2 × 50 mL), and purified by chromatography over silica gel (EtOAc → EtOAc/MeOH, 30/2, v/v) to give (*R*)-**3** (51%) and (*S*)-**3** (69%) as white foams. Compound (*R*)-**3**: $^{1}$H NMR (400 MHz, $CDCl_3$) 3.06 (dd, $^{3}J_{H,H}$ = 6.3 Hz, $^{2}J_{H,H}$ = 9.6 Hz, 1H), 3.28 (dd, $^{3}J_{H,H}$ = 5.4 Hz, $^{2}J_{H,H}$ = 9.6 Hz, 1H), 4.15 – 4.23 (m, 1H), 4.28 (dd, $^{3}J_{H,H}$ = 6.8 Hz, $^{2}J_{H,H}$ = 14.2 Hz, 1H), 4.39 (dd, $^{3}J_{H,H}$ = 2.2 Hz, $^{2}J_{H,H}$ = 14.2 Hz, 1H), 5.85 (br s, 2H), 7.16 – 7.32 (m, 9H), 7.40 (d, $^{3}J_{H,H}$ = 7.2 Hz, 6H), 7.68 (s, 1H), 8.22 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) 48.5, 64.7, 69.7, 87.2, 119.5, 127.4, 128.1, 128.6, 141.8, 143.6, 152.5, 155.5; HRMS *m/z* calcd for $C_{27}H_{26}N_5O_2$ $[M+H]^+$ 452.20810, found 452.20799. Compound (*S*)-**3**: $^{1}$H and $^{13}$C NMR spectra are identical with (*R*)-**3**. HRMS *m/z* calcd for $C_{27}H_{26}N_5O_2$ $[M+H]^+$ 452.20810, found 452.20800.

**(*R*)-*N*-{9-[2-Hydroxy-3-(trityloxy)propyl]-9*H*-purin-6-yl}benzamide (*R*)-4 and (*S*)-*N*-{9-[2-Hydroxy-3-(trityloxy)propyl]-9*H*-purin-6-yl}benzamide (*S*)-4.** In analogy to the reported procedure,[18] compound (*R*)-**3** or (*S*)-**3** (6.77 g, 15 mmol) was disolved in anhydrous pyridine (100 mL) and trimethylsilyl chloride (8 mL, 63 mmol) was added at room temperature. After stirring for 2 h, the mixture was cooled to 0 °C and benzoyl chloride (2.6 mL, 22.5 mmol) was added dropwise. The mixture was allowed to warm to room temperature and was stirred for additional 2 h. Water (20 mL) was added at 0 °C with stirring, and after 15 min, 25% aq. $NH_4OH$ (40 mL) was added. After stirring for another 30 min, the mixture was extracted with EtOAc (2 × 100 mL). Solvents were partially removed *in vacuo* and the products were precipitated to give (*R*)-**4** (51%) and (*S*)-**4** (41%) as white powders. Compound (*R*)-**4**: 1H NMR (400 MHz, $CDCl_3$) 3.16 (dd, $^{3}J_{H,H}$ = 5.7 Hz, $^{2}J_{H,H}$ = 9.7 Hz, 1H), 3.25 (dd, $^{3}J_{H,H}$ = 5.5 Hz, $^{2}J_{H,H}$ = 9.7 Hz, 1H), 4.16 – 4.24 (m, 1H), 4.32 (dd, $^{3}J_{H,H}$ = 7.1 Hz, $^{2}J_{H,H}$ = 14.2 Hz, 1H), 4.49 (dd, $^{3}J_{H,H}$ = 2.2 Hz, $^{2}J_{H,H}$ = 14.2 Hz, 1H), 7.18 – 7.33 (m, 9H), 7.40 (d, $^{3}J_{H,H}$ = 7.3 Hz, 6H), 7.51 (t, $^{3}J_{H,H}$ = 7.5 Hz, 2H), 7.61 (t, $^{3}J_{H,H}$ = 7.4 Hz, 1H), 7.99 – 8.06 (m, 3H), 8.71 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) 48.0, 64.9, 69.4, 87.3, 122.6, 127.4, 128.0, 128.1, 128.6, 128.9, 132.9, 133.8, 143.5, 144.3, 149.5, 152.1, 152.3 164.9; HRMS *m/z* calcd for $C_{34}H_{30}N_5O_3$ $[M+H]^+$ 556.23432, found 556.23435. Compound (*S*)-**4**: $^{1}$H and $^{13}$C NMR spectra are identical with (*R*)-**4**. HRMS *m/z* calcd for $C_{34}H_{30}N_5O_3$ $[M+H]^+$ 556.23432, found 556.23435.

**(*R*)-*N*-{9-[2-Azido-3-(trityloxy)propyl]-9*H*-purin-6-yl}benzamide (*R*)-5 and (*S*)-*N*-{9-[2-Azido-3-(trityloxy)propyl]-9*H*-purin-6-yl}benzamide (*S*)-5.** In analogy to reported procedure,[19] compounds (*R*)-**4** or (*S*)-**4** (4.17 g, 7.5 mmol) was dissolved in anhydrous pyridine (70 mL). The mixture was cooled to 0 °C and methanesulfonyl chloride (1.2 mL, 15 mmol) was added drop-wise. The reaction mixture was then warmed up to room temperature and stirred for 4 h. Methanol (5 mL) was added at 0 °C and the solvents were evaporated *in vacuo*. The crude product was dissolved in $CH_2Cl_2$ (200 mL), the solution was washed with saturated aq. $NaHCO_3$ (2 × 100 mL), with brine (1 × 100 mL) and dried (anhydrous $Na_2SO_4$). After filtration the solvent was evaporated *in vacuo*. The resulting foam was dissolved in a DMF/HMPA mixture (1:1, 20 mL) and sodium azide (2.3 g, 35 mmol) was added. The reaction mixture was stirred at 100 °C for 12 h, cooled down to room temperature and poured into EtOAc (200 mL). The organic layer was washed with water (2 × 200 mL), dried over anhydrous $Na_2SO_4$, filtered, evaporated *in vacuo*, and purified by silica gel chromatography ($CHCl_3$ to $CHCl_3$/MeOH, 98/2, v/v) to afford (*R*)-**5** (80%) and (*S*)-**5** (51%) as white foams. Compound (*R*)-**5**: $^1$H NMR (400 MHz, $CDCl_3$) 3.30 (dd, $^3J_{H,H}$ = 6.3 Hz, $^2J_{H,H}$ = 10.2 Hz, 1H), 3.45 (dd, $^3J_{H,H}$ = 4.2 Hz, $^2J_{H,H}$ = 10.2 Hz, 1H), 3.95 – 4.03 (m, 1H), 4.18 (dd, $^3J_{H,H}$ = 8.6 Hz, $^2J_{H,H}$ = 14.3 Hz, 1H), 4.41 (dd, $^3J_{H,H}$ = 4.1 Hz, $^2J_{H,H}$ = 14.3 Hz, 1H), 7.26 (t, $^3J_{H,H}$ = 7.2 Hz, 3H), 7.33 (t, $^3J_{H,H}$ = 7.4 Hz, 6H), 7.45 (d, $^3J_{H,H}$ = 7.2 Hz, 6H), 7.52 (t, $^3J_{H,H}$ = 7.5 Hz, 2H), 7.60 (t, $^3J_{H,H}$ = 7.4 Hz, 1H), 7.98 (s, 1H), 8.03 (d, $^3J_{H,H}$ = 7.2 Hz, 2H), 8.76 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) 44.8, 60.9, 64.0, 87.8, 122.8, 127.5, 128.0, 128.2, 128.6, 129.0, 132.9, 133.8, 143.3, 143.5, 149.7, 152.1, 152.7, 164.8; HRMS *m/z* calcd for $C_{34}H_{29}N_8O_2$ $[M+H]^+$ 581.24080, found 581.24086. $[\alpha]^{20}_D$ +9.5 (*c* 0.59, MeOH). Compound (*S*)-**5**: $^1$H and $^{13}$C NMR spectra are identical with (*R*)-**5**. HRMS *m/z* calcd for $C_{34}H_{29}N_8O_2$ $[M+H]^+$ 581.24080, found 581.24086. $[\alpha]^{20}_D$ –8.6 (*c* 0.50, MeOH).

**Diethyl (*R*)-1-[1-(6-benzamido-9*H*-purin-9-yl)-3-(trityloxy)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*R*)-6 and Diethyl (*S*)-1-[1-(6-benzamido-9*H*-purin-9-yl)-3-(trityloxy)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*S*)-6.** Compounds (*R*)-**5** and (*S*)-**5** were treated by *GP1* to give (*R*)-**6** (68%) and (*S*)-**6** (83%), respectively, as white powders. Compound (*R*)-**6**: $^1$H NMR (400 MHz, $CDCl_3$) 1.28 and 1.30 (2 × t, $^3J_{H,H}$ = 7.1 Hz, 2 × 3H), 3.67 (dd, $^3J_{H,H}$ = 6.3 Hz, $^2J_{H,H}$ = 10.4 Hz, 1H), 3.71 (dd, $^3J_{H,H}$ = 4.9 Hz, $^2J_{H,H}$ = 10.4 Hz, 1H), 4.15 – 4.35 (m, 4H), 4.86 (dd, $^3J_{H,H}$ = 4.6 Hz, $^2J_{H,H}$ = 14.5 Hz, 1H), 5.01 (dd, $^3J_{H,H}$ = 9.4 Hz, $^2J_{H,H}$ = 14.5 Hz, 1H), 5.13 – 5.22 (m, 1H), 7.17 – 7.34 (m, 15H), 7.50 (t, $^3J_{H,H}$ = 7.6 Hz, 2H), 7.59 (t, $^3J_{H,H}$ = 7.4 Hz, 1H), 7.71 (s, 1H), 7.95 (s, 1H), 8.03 (d, $^3J_{H,H}$ = 7.2 Hz, 2H), 8.74 (s,

1H); $^{13}$C NMR (100 MHz, $CDCl_3$) 16.3 (d, $^3J_{C,P}$ = 6.4 Hz), 44.6, 60.7, 63.2 and 63.3 (2 × d, $^2J_{C,P}$ = 5.8 Hz), 63.9, 88.0, 122.7, 127.7, 128.0, 128.3, 128.4, 129.0, 131.6 (d, $^2J_{C,P}$ = 32.8 Hz), 133.0, 133.7, 137.9 (d, $^1J_{C,P}$ = 238.5 Hz), 142.9, 143.1, 149.8, 151.9, 152.8, 164.8; $^{31}$P (161.9 MHz, $CDCl_3$) 6.57; HRMS *m/z* calcd for $C_{40}H_{39}N_8NaO_5P$ $[M+Na]^+$ 765.26732, found 765.26705. $[\alpha]^{20}_D$ +36.0 (*c* 0.56, MeOH). Compound (*S*)-**6**: $^1$H and $^{13}$C NMR spectra are identical with (*R*)-**6**. HRMS *m/z* calcd for $C_{40}H_{39}N_8NaO_5P$ $[M+Na]^+$ 765.26732, found 765.26727. $[\alpha]^{20}_D$ –34.0 (*c* 0.52, MeOH).

**Diethyl (*R*)-1-[1-(6-amino-9H-purin-9-yl)-3-hydroxypropan-2-yl)-1*H*-1,2,3-triazol-4-ylphosphonate (*R*)-7 and Diethyl (*S*)-1-[1-(6-amino-9H-purin-9-yl)-3-hydroxypropan-2-yl)-1*H*-1,2,3-triazol-4-ylphosphonate (*S*)-7.** In analogy to the reported procedure,[21] methylamine in MeOH (4 mL, 8M solution) was added dropwise at room temperature to a solution of (*R*)-**6** or (*S*)-**6** (1.11 g, 1.5 mmol) in anhydrous toluene (20 mL). The reaction mixture was stirred for 4 h at room temperature, volatiles were evaporated and the residue was disolved in 80% aq. acetic acid (20 mL). The mixture was heated at 90 °C for 4 h, cooled down to room temperature and solvents were evaporated. Column chromatography over silica gel ($CHCl_3$ → $CHCl_3$/MeOH, 100/20, v/v) afforded (*R*)-**7** (74%) and (*S*)-**7** (70%) as colourless solid. Compound (*R*)-**7**: $^1$H NMR (400 MHz, DMSO-*d6*) 1.18 and 1.19 (2 × t, $^3J_{H,H}$ = 6.9 Hz, 2 × 3H), 3.84 – 4.02 (m, 6H), 4.67 (dd, $^3J_{H,H}$ = 4.8 Hz, $^2J_{H,H}$ = 14.5 Hz, 1H), 4.75 (dd, $^3J_{H,H}$ = 9.7 Hz, $^2J_{H,H}$ = 14.5 Hz, 1H), 5.27 – 5.37 (m, 1H), 5.44 (t, $^3J_{H,H}$ = 5.4 Hz, 1H), 7.22 (br s, 2H), 7.85 (s, 1H), 8.06 (s, 1H), 8.68 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-*d6*) 28.4 and 28.5 (2 × d, $^3J_{C,P}$ = 6.2 Hz), 56.1, 73.2, 74.6 and 74.7 (2 × d, $^2J_{C,P}$ = 5.6 Hz), 74.9, 130.8, 143.5 (d, $^2J_{C,P}$ = 33.6 Hz), 148.5 (d, $^1J_{C,P}$ = 236.6 Hz), 152.9, 161.9, 164.9, 168.4; $^{31}$P NMR (161.9 MHz, $CDCl_3$) 8.31; HRMS *m/z* calcd for $C_{14}H_{22}N_8O_4P$ $[M+H]^+$ 397.14961, found 397.14949. $[\alpha]^{20}_D$ +101.9 (*c* 0.59, MeOH). Compound (*S*)-**7**: $^1$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**7**. HRMS *m/z* calcd for $C_{14}H_{22}N_8O_4P$ $[M+H]^+$ 397.14961, found 397.14944. $[\alpha]^{20}_D$ –102.7 (*c* 0.59, MeOH).

**(*R*)-1-[1-(6-Amino-9*H*-purin-9-yl)-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonic acid (*R*)-8 and (*S*)-1-[1-(6-Amino-9*H*-purin-9-yl)-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonic acid (*S*)-8.** Compounds (*R*)-**7** and (*S*)-**7** were treated by *GP2* to give (*R*)-**8** (80%) and (*S*)-**8** (70%), respectively, as white powders. Compound (*R*)-**8**: $^1$H NMR (400 MHz, DMSO-*d6*) 3.88 (d, $^3J_{H,H}$ = 5.5 Hz, 2H), 4.69 (dd, $^3J_{H,H}$ = 4.9 Hz, $^2J_{H,H}$ = 14.5 Hz, 1H), 4.78 (dd, $^3J_{H,H}$ = 9.5 Hz, $^2J_{H,H}$ = 14.5 Hz, 1H), 5.25 – 5.35 (m, 1H), 7.44 (br s,

2H), 7.79 (s, 1H), 8,15 (s, 1H), 8.44 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) 43.9, 61.2, 61.6, 118.4, 128.6 (d, $^{2}J_{C,P}$ = 32.4 Hz), 140.8, 141.6 (d, $^{1}J_{C,P}$ = 228.7 Hz), 149.4, 151.9, 155.5; $^{31}$P NMR (161.9 MHz, $CDCl_3$) 3.17; HRMS *m/z* calcd for $C_{10}H_{12}N_8O_4P$ $[M-H]^-$ 339.07246, found 339.07190. $[\alpha]^{20}_D$ +95.2 (*c* 0.52, $H_2O$). Compound (*S*)-**8**: $^{1}$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**8**. HRMS *m/z* calcd for $C_{10}H_{12}N_8O_4P$ $[M-H]^-$ 339.07246, found 339.07224. $[\alpha]^{20}_D$ –89.2 (*c* 0.51, $H_2O$).

**(*R*)-1-Benzyl-2-mesyl-3-tritylglycerol (*R*)-9 and (*S*)-1-Benzyl-2-mesyl-3-tritylglycerol (*S*)-9.** 60% NaH in mineral oil (190 mmol, 7.6 g) was suspended in anhydrous DMF (200 mL) and benzyl alcohol (20 mL, 190 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 20 min and a mixture of tritylated glycidol (*R*)-**2** or (*S*)-**2** (158 mmol, 50 g) in DMF (100 mL) was added. The mixture was stirred at 100 °C for 2 h, cooled down to room temperature and diluted with water (50 mL). The mixture was extracted with EtOAc (3 × 250 mL). Joint organic portions were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to afford (*R*)-1-benzyl-3-tritylglycerol and (*S*)-1-benzyl-3-tritylglycerol, respectively. Each crude intermediate was dissolved in anhydrous pyridine (200 mL) and methanesulfonyl chloride (40 mL) was added dropwise at 0 °C. The reaction mixture was then stirred at room temperature for 4 h. Methanol (50 mL) was added at 0 °C and solvents were evaporated *in vacuo*. The residue was dissolved in $CH_2Cl_2$ (500 mL) and washed with $H_2O$ (200 mL), solution of sat. $NaHCO_3$ (2 × 200 mL), and brine (50 mL). The organic solution was dried (anhydrous $Na_2SO_4$), filtered, and concentrated *in vacuo*. Precipitation from a $Et_2O$/hexane (10/3, v/v) mixture gave (*R*)-**9** (68%) and (*S*)-**9** (63%) as white powders. Compound (*R*)-**9**: $^{1}$H NMR (400 MHz, $CDCl_3$) 3.03 (s, 3H), 3.37 (dd, $^{3}J_{H,H}$ = 5.6 Hz, $^{2}J_{H,H}$ = 10.6 Hz, 1H), 3.43 (dd, $^{3}J_{H,H}$ = 4.3 Hz, $^{2}J_{H,H}$ = 10.6 Hz, 1H), 3.68 (dd, $^{3}J_{H,H}$ = 3.9 Hz, $^{2}J_{H,H}$ = 10.8 Hz, 1H), 3.75 (dd, $^{3}J_{H,H}$ = 6.8 Hz, $^{2}J_{H,H}$ = 10.8 Hz, 1H), 4.53 (s, 2H), 4.86 – 4.93 (m, 1H), 7.23 – 7.48 (m, 20H); $^{13}$C NMR (100 MHz, $CDCl_3$) 38.7, 63.3, 69.6, 73.5, 80.8, 87.3, 127.4, 127.9, 128.0, 128.1, 128.6, 128.7, 137.6, 143.5; HRMS *m/z* calcd for $C_{30}H_{30}NaO_5S$ $[M+Na]^+$ 525.17062, found 525.17078. $[\alpha]^{20}_D$ +6.7 (*c* 1.12, $CHCl_3$). Compound (*S*)-**9**: $^{1}$H and $^{13}$C NMR spectra are identical with (*R*)-**9**. HRMS *m/z* calcd for $C_{30}H_{30}NaO_5S$ $[M+Na]^+$ 525.17062, found 525.17078. $[\alpha]^{20}_D$ –5.1 (*c* 1.65, $CHCl_3$).

**(*R*)-2-Azido-3-benzyloxy-1-(trityloxy)propane (*R*)-10 and (*S*)-2-Azido-3-benzyloxy-1-(trityloxy)propane (*S*)-10.** According to the reported procedure,[19] sodium azide (300 mmol, 19.5 g) was added to the mixture of compound (*R*)-**9** or (*S*)-**9** (0.1 mol, 50.3 g) in

HMPA/DMF (200 mL, 1:1) and the mixture was stirred at 100 °C for 5h and then diluted with EtOAc (500 mL). The organic phase was washed with water (2 × 400 mL), dried (anhydrous $Na_2SO_4$), filtered, and evaporated *in vacuo*. A column chromatography over silica gel (hexane → hexane/EtOAc, 90/10, v/v) afforded (*R*)-**10** (99%) and (*S*)-**10** (97%) as colourless oils. Compound (*R*)-**10**: $^1$H NMR (400 MHz, $CDCl_3$) 3.26 (dd, $^3J_{H,H}$ = 6.2 Hz, $^2J_{H,H}$ = 9.8 Hz, 1H), 3.29 (dd, $^3J_{H,H}$ = 4.8 Hz, $^2J_{H,H}$ = 9.8 Hz, 1H), 3.57 (dd, $^3J_{H,H}$ = 6.8 Hz, $^2J_{H,H}$ = 9.9 Hz, 1H), 3.61 (dd, $^3J_{H,H}$ = 4.5 Hz, $^2J_{H,H}$ = 9.9 Hz, 1H), 4.53 (s, 2H), 7.22 – 7.38 (m, 15H), 7.42 – 7.48 (m, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) 61.4, 63.5, 70.0, 73.5, 87.3, 127.3, 127.7, 127.9, 128.0, 128.5, 128.8, 137.9, 143.8; HRMS *m/z* calcd for $C_{29}H_{27}N_3NaO_2$ $[M+Na]^+$ 472.19955, found 472.19951. $[\alpha]^{20}_D$ –1.4 (*c* 2.58, MeOH). Compound (*S*)-**10**: $^1$H and $^{13}$C NMR spectra are identical with (*R*)-**10**. HRMS *m/z* calcd for $C_{29}H_{27}N_3NaO_2$ $[M+Na]^+$ 472.19955, found 472.19953. $[\alpha]^{20}_D$ +1.4 (*c* 2.19, MeOH).

**Diethyl (*R*)-1-[-1hydroxy-3-(trityloxy)propan-2-yl]-*1H*-1,2,3-triazol-4-ylphosphonate (*R*)-12 and Diethyl (*S*)-1-[-1hydroxy-3-(trityloxy)propan-2-yl]-*1H*-1,2,3-triazol-4-ylphosphonate (*S*)-12.** In analogy to the reported procedure,[24] to a solution of compound (*R*)-**10** or (*S*)-**10** (27.0 g, 60.0 mmol) in EtOAc (200 mL) was added a solution of $NaBrO_3$ (27.2 g, 0.18 mol) in water (200 mL). A solution of $Na_2S_2O_4$ (31.3 g, 0.18 mol) in water (200 mL) was added to the reaction mixture over 1 h and the mixture was vigorously stirred for 2.5 h at room temperature. The mixture was diluted with EtOAc (150 mL), quenched with 10% $Na_2S_2O_3$ (50 mL) and extracted with water (3 × 150 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography (hexane → hexane/EtOAc, 80/20, v/v) to give (*R*)-**11** (89%) and (*S*)-**11** (91%), respectively, as yellowish oils. Treatment of crude compound (*R*)-**11** or (*S*)-**11** by *GP1* afforded compounds (*R*)-**12** (87%) and (*S*)-**12** (73%) as colourless solid. Compound (*R*)-**12**: $^1$H (400 MHz, $CDCl_3$) 1.27 and 1.28 (2 × t, $^3J_{H,H}$ = 7.1 Hz, 2 × 3H), 3.58 (dd, $^3J_{H,H}$ = 5.1 Hz, $^2J_{H,H}$ = 10.1 Hz, 1H), 3.64 (dd, $^3J_{H,H}$ = 7.1 Hz, $^2J_{H,H}$ = 10.1 Hz, 1H), 3.96 – 4.19 (m, 7H), 4.76 – 4.86 (m, 1H), 7.17 – 7.30 (m, 15H), 8.20 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) 16.3 (2 × d, $^3J_{C,P}$ = 6.2 Hz), 61.9, 63.0, 63.3 and 63.5 (2 × d, $^2J_{C,P}$ = 5.9 Hz), 63.4, 87.4, 127.5, 128.1, 128.5, 131.4 (d, $^2J_{C,P}$ = 33.4 Hz), 136.6 (d, $^1J_{C,P}$ = 239.7 Hz), 143.2; HRMS *m/z* calcd for $C_{28}H_{32}N_3NaO_5P$ $[M+Na]^+$ 544.19718, found 544.19706. $[\alpha]^{20}_D$ –10.1 (*c* 1.32, MeOH). Compound (*S*)-**12**: $^1$H and $^{13}$C NMR spectra are identical with (*R*)-**12**. HRMS *m/z* calcd for $C_{28}H_{32}N_3NaO_5P$ $[M+Na]^+$ 544.19718, found 544.19705. $[\alpha]^{20}_D$ +10.9 (*c* 1.44, MeOH).

**Diethyl (*R*)-1-[1-(6-chloro-9*H*-purin-9-yl)-3-(trityloxy)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*R*)-13 and Diethyl (*S*)-1-[1-(6-chloro-9*H*-purin-9-yl)-3-(trityloxy)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*S*)-13.** Treatment of (*R*)-**12** or (*S*)-**12** with 6-chloropurine by *GP3*, followed by purification by column chromatography over silica gel ($CHCl_3$/MeOH, 98/2, v/v), afforded (*R*)-**13** (64%) and (*S*)-**13** (67%), respectively, as colourless foams. Compound (*R*)-**13**: $^1$H NMR (400 MHz, $CDCl_3$) 1.29 and 1.30 (2 × t, $^3J_{H,H}$ = 7.1 Hz, 2 × 3H), 3.62 – 3.73 (m, 2H), 4.04 – 4.22 (m, 4H), 4.87 (dd, $^3J_{H,H}$ = 4.8 Hz, $^2J_{H,H}$ = 14.4 Hz, 1H), 5.02 (dd, $^3J_{H,H}$ = 9.2 Hz, $^2J_{H,H}$ = 14.4 Hz, 1H), 5.08 – 5.17 (m, 1H), 7.19 – 7.35 (m, 15H), 7.82 (s, 1H), 7.94 (s, 1H), 8.69 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) 16.4 (d, $^3J_{C,P}$ = 6.5 Hz), 44.6, 60.5, 63.3 (d, $^2J_{C,P}$ = 5.7 Hz), 63.6, 88.0, 127.7, 128.3, 128.4, 131.5 (d, $^2J_{C,P}$ = 33.0 Hz), 131.6, 138.0 (d, $^1J_{C,P}$ = 238.3 Hz), 142.8, 145.4, 147.0, 151.6, 152.2; $^{31}$P NMR (161.9 MHz, $CDCl_3$) 6.23; HRMS *m/z* calcd for $C_{33}H_{33}ClN_7NaO_4P$ $[M+Na]^+$ 680.19124, found 680.19119. $[\alpha]^{20}_D$ +30.0 (*c* 0.26, MeOH). Compound (*S*)-**13**: $^1$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**13**. HRMS *m/z* calcd for $C_{33}H_{33}ClN_7NaO_4P$ $[M+Na]^+$ 680.19124, found 680.19125. $[\alpha]^{20}_D$ –26.3 (*c* 0.32, MeOH).

**Diethyl (*R*)-1-[1-hydroxy-3-(6-oxo-1*H*-purin-9(6*H*)-yl)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*R*)-14 and Diethyl (*S*)-1-[1-hydroxy-3-(6-oxo-1*H*-purin-9(6*H*)-yl)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*S*)-14.** A mixture of (*R*)-**13** or (*S*)-**13** (2.3 mmol) in 75% aq. trifluoroacetic acid (20 mL) was stirred at room temperature for 48 h. Solvents were evaporated and the crude product was purified by chromatography over silica gel ($CHCl_3$/MeOH, 95/5, v/v → $CHCl_3$/MeOH, 80/20, v/v). Crystallization from a mixture of MeOH/$Et_2O$ (1:1) gave (*R*)-**14** (80%) and (*S*)-**14** (91%) as colourless crystals. Compound (*R*)-**14**: mp 185 °C; $^1$H NMR (400 MHz, DMSO-*d6*) 1.19 and 1.20 (2 × t, $^3J_{H,H}$ = 7.0 Hz, 2 × 3H), 3.86 – 4.06 (m, 6H), 4.63 – 4.78 (m, 2H), 5.20 – 5.30 (m, 1H), 5.44 (t, $^3J_{H,H}$ = 5.4 Hz, 1H), 7.82 (s, 1H), 7.95 (s, 1H), 8.66 (s, 1H), 12.30 (br s, 1H); $^{13}$C NMR (100 MHz, DMSO-*d6*) 16.0 (d, $^3J_{C,P}$ = 6.2 Hz), 44.1, 60.6, 62.1 and 62.2 (2 × d, $^2J_{C,P}$ = 5.6 Hz), 62.6, 123.6, 131.1 (d, $^2J_{C,P}$ = 33.6 Hz), 136.1 (d, $^1J_{C,P}$ = 236.9 Hz), 140.0, 145.5, 148.3, 156.4; $^{31}$P NMR (161.9 MHz, DMSO-*d6*) 8.32; HRMS *m/z* calcd for $C_{14}H_{20}N_7NaO_5P$ $[M+Na]^+$ 420.11557, found 420.11561. $[\alpha]^{20}_D$ +101.1 (*c* 0.27, MeOH). Compound (*S*)-**14**: mp 185 °C; $^1$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**14**. HRMS *m/z* calcd for $C_{14}H_{20}N_7NaO_5P$ $[M+Na]^+$ 420.11557, found 420.11547. $[\alpha]^{20}_D$ –97.0 (*c* 0.36, MeOH).

**(*R*)-1-[1-Hydroxy-3-(6-oxo-1*H*-purin-9(6*H*)-yl)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonic acid (*R*)-15 and (*S*)-1-[1-Hydroxy-3-(6-oxo-1*H*-purin-9(6*H*)-yl)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonic acid (*S*)-15.** Treatment of (*R*)-**14** or (*S*)-**14** by *PG2* afforded (*R*)-**15** (54%) and (*S*)-**15** (59%), respectively, as white amorphous powders. Compound (*R*)-**15**: $^{1}$H NMR (400 MHz, DMSO-*d6*) 3.91 (d, $^{3}J_{H,H}$ = 5.6 Hz, 2H), 4.69 (dd, $^{3}J_{H,H}$ = 4.8 Hz, $^{2}J_{H,H}$ = 14.5 Hz, 1H), 4.76 (dd, $^{3}J_{H,H}$ = 9.5 Hz, $^{2}J_{H,H}$ = 14.5 Hz, 1H), 5.18 – 5.28 (m, 1H), 7.79 (s, 1H), 8.00 (s, 1H), 8.43 (s, 1H), 12.35 (br s, 1H); $^{13}$C NMR (100 MHz, DMSO-*d6*) 44.2, 61.0, 61.9, 123.5, 128.8 (d, $^{2}J_{C,P}$ = 32.2 Hz), 140.0, 141.3 (d, $^{1}J_{C,P}$ = 229.4 Hz), 145.7, 148.3, 156.4; $^{31}$P NMR (161.9 MHz, DMSO-*d6*) 3.40; HRMS *m/z* calcd for $C_{10}H_{11}N_7O_5P$ $[M-H]^-$ 340.05648, found 340.05634. $[\alpha]^{20}_D$ +80.5 (*c* 0.33, $H_2O$). Compound (*S*)-**15**: $^{1}$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**15**; HRMS *m/z* calcd for $C_{10}H_{11}N_7O_5P$ $[M-H]^-$ 340.05648, found 340.05665. $[\alpha]^{20}_D$ –79.3 (*c* 0.24, $H_2O$).

**Diethyl (*R*)-1-[1-(2-amino-6-chloro-9*H*-purin-9-yl)-3-(trityloxy)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*R*)-16 and Diethyl (*S*)-1-[1-(2-amino-6-chloro-9*H*-purin-9-yl)-3-(trityloxy)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*S*)-16.** Treatment of (*R*)-**12** or (*S*)-**12** with 2-amino-6-chloropurine by *GP3*, followed by purification by column chromatography over silica gel (hexane/$CHCl_3$/MeOH, 6/4/0.1 → hexane/$CHCl_3$/MeOH, 6/4/0.3), afforded (*R*)-**16** (31%) and (*S*)-**16** (46%), respectively, as whitish foams. Compound (*R*)-**16**: $^{1}$H NMR (400 MHz, $CDCl_3$) 1.30 and 1.31 (2 × t, $^{3}J_{H,H}$ = 7.2 Hz, 2 × 3H), 3.65 (d, $^{3}J_{H,H}$ = 5.6 Hz, 2H), 4.06 – 4.22 (m, 4H), 4.63 (dd, $^{3}J_{H,H}$ = 4.9 Hz, $^{2}J_{H,H}$ = 14.6 Hz, 1H), 4.79 (dd, $^{3}J_{H,H}$ = 9.0 Hz, $^{2}J_{H,H}$ = 14.6 Hz, 1H), 5.02 – 5.11 (m, 1H), 5.21 (br s, 2H), 7.21 – 7.32 (m, 15H), 7.45 (s, 1H), 8.08 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) 16.4 (d, $^{3}J_{C,P}$ = 6.5 Hz), 44.5, 60.6, 63.3 (d, $^{2}J_{C,P}$ = 5.7 Hz), 63.6, 88.0, 125.1, 127.7, 128.3, 128.5, 131.6 (d, $^{2}J_{C,P}$ = 33.0 Hz), 137.8 (d, $^{1}J_{C,P}$ = 238.1 Hz), 142.1, 142.9, 151.8, 153.6, 159.2; $^{31}$P NMR (161.9 MHz, $CDCl_3$) 6.58; HRMS *m/z* calcd for $C_{33}H_{34}N_8NaO_4P$ $[M+Na]^+$ 695.20214, found 695.20209. $[\alpha]^{20}_D$ +36.2 (*c* 0.41, MeOH). Compound (*S*)-**16**: $^{1}$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**16**. HRMS *m/z* calcd for $C_{33}H_{34}N_8NaO_4P$ $[M+Na]^+$ 695.20214, found 695.20212. $[\alpha]^{20}_D$ –36.3 (*c* 0.38, MeOH).

**Diethyl (*R*)-1-[1-(2-amino-6-oxo-1*H*-purin9(6*H*)-yl)-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*R*)-17 and Diethyl (*S*)-1-[1-(2-amino-6-oxo-1*H*-purin9(6*H*)-yl)-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*S*)-17.** A mixture of (*R*)-**16** or (*S*)-**16** (2.3 mmol) in 75% aq. trifluoroacetic acid (20 mL) was stirred at room temperature for

48 h. Solvents were evaporated and the crude product was purified by chromatography over silica gel ($CHCl_3$/MeOH, 98/2 → $CHCl_3$/MeOH, 80/30) to give (*R*)-**17** (90%) and (*S*)-**17** (91%), respectively, as white amorphous solids. Compound (*R*)-**17**: $^1$H NMR (400 MHz, DMSO-*d6*) 1.20 and 1.21 (2 × t, $^3J_{H,H}$ = 7.1 Hz, 2 × 3H), 3.86 – 4.05 (m, 6H), 4.48 (dd, $^3J_{H,H}$ = 5.0 Hz, $^2J_{H,H}$ = 14.5 Hz, 1H), 4.56 (dd, $^3J_{H,H}$ = 9.7 Hz, $^2J_{H,H}$ = 14.5 Hz, 1H), 5.18 – 5.28 (m, 1H), 5.36 – 5.57 (m, 1H), 6.55 (br s, 2H), 7.34 (s, 1H), 8.66 (s, 1H), 10.67 (br s, 1H); $^{13}$C NMR (100 MHz, DMSO-*d6*) 16.0 (d, $^3J_{C,P}$ = 6.2 Hz), 43.4, 60.7, 62.1, 62.2 and 62.3 (2 × d, $^2J_{C,P}$ = 5.6 Hz), 116.2, 131.1 (d, $^2J_{C,P}$ = 33.7 Hz), 136.0 (d, $^1J_{C,P}$ = 236.8 Hz), 136.9, 151.1, 153.7, 156.6; $^{31}$P NMR (161.9 MHz, DMSO-*d6*) 8.39; HRMS *m/z* calcd for $C_{14}H_{21}N_8NaO_5P$ $[M+Na]^+$ 435.12647, found 435.12651. $[\alpha]^{20}_D$ +72.7 (*c* 0.97, MeOH). Compound (*S*)-**17**: $^1$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**17**; HRMS *m/z* calcd for $C_{14}H_{21}N_8NaO_5P$ $[M+Na]^+$ 435.12647, found 435.12644. $[\alpha]^{20}_D$ –82.4 (*c* 0.95, MeOH).

**(*R*)-1-[1-(2-Amino-6-oxo-1*H*-purin9(6*H*)-yl)-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonic acid (*R*)-18 and (*S*)-1-[1-(2-Amino-6-oxo-1*H*-purin9(6*H*)-yl)-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonic acid (*S*)-18.** Treatment of (*R*)-**17** or (*S*)-**17** by *PG2* afforded (*R*)-**18** (59%) and (*S*)-**18** (86%), respectively, as white amorphous powders. Compound (*R*)-**18**: $^1$H NMR (400 MHz, DMSO-*d6*) 3.82 (d, $^3J_{H,H}$ = 5.5 Hz, 2H), 4.48 (dd, $^3J_{H,H}$ = 5.9 Hz, $^2J_{H,H}$ = 14.5 Hz, 1H), 4.57 (dd, $^3J_{H,H}$ = 9.3 Hz, $^2J_{H,H}$ = 14.5 Hz, 1H), 5.14 – 5.23 (m, 1H), 5.30 – 5.50 (m, 1H), 6.48 (br s, 1H), 7.30 (s, 1H), 8.45 (s, 1H), 10.59 (br s, 1H); $^{13}$C NMR (100 MHz, DMSO-*d6*) 43.9, 61.0, 61.3, 116.3, 128.9 (d, $^2J_{C,P}$ = 32.1 Hz), 137.1, 141.4 (d, $^1J_{C,P}$ = 229.4 Hz), 150.9, 154.0, 155.9; $^{31}$P NMR (161.9 MHz, DMSO-*d6*) 3.56; HRMS *m/z* calcd for $C_{10}H_{12}N_8O_5P$ $[M-H]^-$ 355.06738, found 355.06726. $[\alpha]^{20}_D$ +92.7 (*c* 0.23, $H_2O$). Compound (*S*)-**18**: $^1$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**18**; HRMS *m/z* calcd for $C_{10}H_{12}N_8O_5P$ $[M-H]^-$ 355.06738, found 355.06726. $[\alpha]^{20}_D$ –87.7 (*c* 0.25, $H_2O$).

**(*R*)-1-[1-(2,6-Dioxo-2,3-dihydro-1*H*-purin-9(6*H*)-yl)-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonic acid (*R*)-19 and (*S*)-1-[1-(2,6-Dioxo-2,3-dihydro-1*H*-purin-9(6*H*)-yl)-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonic acid (*S*)-19.** Isoamyl nitrite (1.74 g, 2.0 mL) was added to a solution of (*R*)-**18** or (*S*)-**18** (0.4 mmol) in 80% acetic acid (50 mL) and the reaction mixture was stirred at room temperature overnight. Solvents were evaporated and the residues were crystallized from a water-methanol-acetone mixture to offer (*R*)-**19** (45%) and (*S*)-**19** (45%), respectively, as yellowish crystals. Compound (*R*)-**19**: not melting up to 265 °C; $^1$H NMR (400 MHz, DMSO-*d6*) 3.76 – 3.90 (m, 2H), 4.51 (dd, $^3J_{H,H}$ =

4.9 Hz, $^{2}J_{H,H}$ = 14.8 Hz, 1H), 4.68 (dd, $^{3}J_{H,H}$ = 9.9 Hz, $^{2}J_{H,H}$ = 14.8 Hz, 1H), 5.02 – 5.10 (m, 1H), 7.19 (s, 1H), 8.43 (s, 1H), 10.84 (br s, 1H), 12.02 (br s, 1H); $^{13}$C NMR (100 MHz, DMSO-*d6*) 44.2, 60.7, 61.6, 115.2, 129.2 (d, $^{2}J_{C,P}$ = 32.1 Hz), 136.5, 140.2, 141.3 (d, $^{1}J_{C,P}$ = 228.6 Hz), 150.7, 157.7; $^{31}$P NMR (161.9 MHz, DMSO-*d6*) 3.38; HRMS *m/z* calcd for $C_{10}H_{11}N_7O_6P$ $[M-H]^-$ 356.05139, found 356.05141. $[\alpha]^{20}_D$ +70.0 (*c* 0.08, $H_2O$). Compound (*S*)-**19**: not melting up to 265 °C; $^{1}$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**19**; HRMS *m/z* calcd for $C_{10}H_{11}N_7O_6P$ $[M-H]^-$ 356.05139, found 356.05109. $[\alpha]^{20}_D$ –81.5 (*c* 0.07, $H_2O$).

**Diethyl (*R*)-1-[1-(2,6-diamino-9*H*-purin-9-yl)-3-(trityloxy)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*R*)-20 and Diethyl (*S*)-1-[1-(2,6-diamino-9*H*-purin-9-yl)-3-(trityloxy)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*S*)-20.** A mixture of (*R*)-**16** or (*S*)-**16** (1.0 mmol) was dissolved in the ethanolic solution of $NH_3$ (3.5 M, 65 mL) and the reaction mixture was heated in an autoclave at 100 °C for 24 h. Volatiles were evaporated and the residues were purified by flash chromatography over silica gel (hexane/$CHCl_3$/MeOH, 6/4/0.2 → hexane/$CHCl_3$/MeOH, 6/4/1) to give (*R*)-**20** (32%) and (*S*)-**20** (34%), respectively, as yellowish foams. Compound (*R*)-**20**: $^{1}$H NMR (400 MHz, $CDCl_3$) 1.26 and 1.30 (2 × t, $^{3}J_{H,H}$ = 7.1 Hz, 2 × 3H), 3.57 – 3.67 (m, 2H), 4.00 – 4.21 (m, 4H), 4.54 (dd, $^{3}J_{H,H}$ = 4.8 Hz, $^{2}J_{H,H}$ = 14.4 Hz, 1H), 4.72 (dd, $^{3}J_{H,H}$ = 9.2 Hz, $^{2}J_{H,H}$ = 14.4 Hz, 1H), 4.89 (s, 2H), 5.16 – 5.25 (m, 1H), 5.64 (s, 1H), 7.13 (s, 1H), 7.18 – 7.31 (m, 15H), 8.16 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) 16.4 (d, $^{3}J_{C,P}$ = 6.5 Hz), 44.0, 60.8, 63.2 (d, $^{2}J_{C,P}$ = 5.6 Hz), 63.6, 87.8, 114.3, 127.6, 128.2, 128.5, 132.2 (d, $^{2}J_{C,P}$ = 33.2 Hz), 137.4 (d, $^{1}J_{C,P}$ = 237.8 Hz), 137.8, 142.9, 151.9, 156.1, 160.1; $^{31}$P NMR (161.9 MHz, $CDCl_3$) 6.63; HRMS *m/z* calcd for $C_{33}H_{36}N_9NaO_4P$ $[M+Na]^+$ 676.25201, found 676.25163. $[\alpha]^{20}_D$ –45.0 (*c* 0.14, MeOH). Compound (*S*)-**20**: $^{1}$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**20**; HRMS *m/z* calcd for $C_{33}H_{36}N_9NaO_4P$ $[M+Na]^+$ 676.25201, found 676.25168. $[\alpha]^{20}_D$ +50.0 (*c* 0.20, MeOH).

**Diethyl (*R*)-1-[1-(2,6-diamino-9*H*-purin-9-yl)-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*R*)-21 and Diethyl (*S*)-1-[1-(2,6-diamino-9*H*-purin-9-yl)-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*S*)-21.** Treatment of (*R*)-**20** or (*S*)-**20** by *GP4*, followed by purification by chromatography over silica gel ($CHCl_3$/MeOH, 98/2 → $CHCl_3$/MeOH, 70/30) gave (*R*)-**21** (96%) and (*S*)-**21** (97%), respectively, as white amorphous solids. Compound (*R*)-**21**: $^{1}$H NMR (400 MHz, DMSO-*d6*) 1.20 and 1.21 (2 × t, $^{3}J_{H,H}$ = 7.0 Hz, 2 × 3H), 3.83 – 4.07 (m, 6H), 4.49 (dd, $^{3}J_{H,H}$ = 5.1 Hz, $^{2}J_{H,H}$ = 14.5 Hz, 1H),

4.58 (dd, $^{3}J_{H,H}$ = 9.5 Hz, $^{2}J_{H,H}$ = 14.5 Hz, 1H), 5.22 – 5.31 (m, 1H), 5.81 (s, 2H), 6.67 (s, 2H), 7.33 (s, 1H), 8.68 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-*d6*) 16.0 (d, $^{3}J_{C,P}$ = 6.1 Hz), 43.1, 60.8, 62.0, 62.2 and 62.3 (2 × d, $^{2}J_{C,P}$ = 5.7 Hz), 112.8, 131.0 (d, $^{2}J_{C,P}$ = 33.6 Hz), 136.0 (d, $^{1}J_{C,P}$ = 237.0 Hz), 136.9, 151.6, 156.0, 160,3; $^{31}$P NMR (161.9 MHz, DMSO-*d6*) 8.18; HRMS *m/z* calcd for $C_{14}H_{22}N_9NaO_4P$ $[M+Na]^+$ 434.14246, found 434.14229. $[\alpha]^{20}_D$ +34.0 (*c* 0.21, $H_2O$). Compound (*S*)-**21**: $^{1}$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**21**; HRMS *m/z* calcd for $C_{14}H_{23}N_9O_4P$ $[M+H]^+$ 412.16051, found 412.16057. $[\alpha]^{20}_D$ –29.0 (*c* 0.17, $H_2O$).

**(*R*)-1-[1-(2,6-Diamino-9*H*-purin-9-yl)-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonic acid (*R*)-22 and (*S*)-1-[1-(2,6-Diamino-9*H*-purin-9-yl)-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonic acid (S)-22.** Treatment of (*R*)-**21** or (*S*)-**21** by *PG2* afforded (*R*)-**22** (74%) and (*S*)-**22** (86%), respectively, as colorless solids. Compound (*R*)-**22**: $^{1}$H NMR (400 MHz, $D_2O$+NaOD) 4.06 – 4.20 (m, 2H), 4.53 – 4.64 (m, 2H), 5.09 – 5.20 (m, 1H), 7.30 (s, 1H), 7.97 (s, 1H); $^{13}$C NMR (100 MHz, $D_2O$+NaOD) 44.7, 61.3, 62.6, 113.3, 127.8 (d, $^{2}J_{C,P}$ = 26.5 Hz), 140.2, 148.4 (d, $^{1}J_{C,P}$ = 201.6 Hz), 151.5, 156.6, 160.6; $^{31}$P NMR (161.9 MHz, $D_2O$+NaOD) 0.44; HRMS *m/z* calcd for $C_{10}H_{13}N_9O_4P$ $[M-H]^-$ 354.08226, found 354.08260. $[\alpha]^{20}_D$ +166.2 (*c* 0.18, 0.1 M aq. solution of NaOH). Compound (*S*)-**22**: $^{1}$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**22**; HRMS *m/z* calcd for $C_{10}H_{13}N_9O_4P$ $[M-H]^-$ 354.08226, found 354.08194. $[\alpha]^{20}_D$ –162.1 (*c* 0.18, 0.1 M aq. solution of NaOH).

**Diethyl (*R*)-1-[1-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-(trityloxy)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*R*)-23 and Diethyl (*S*)-1-[1-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-(trityloxy)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*S*)-23.** Treatment of (*R*)-**12** or (*S*)-**12** with $N^3$-benzoyluracil by *GP3*, followed by column chromatography over silica gel, afforded crude $N^3$-benzoyluracil intermediates. Propylamine (3 mL) was added to the solutions of the crude intermediates in dioxane (30 mL) and the reaction mixture was stirred at room temperature for 12 h. Column chromatography over silica gel (hexane/$CHCl_3$/MeOH, 6/4/0.1 → hexane/$CHCl_3$/MeOH, 6/4/0.35) afforded (*R*)-**23** (81%) and (*S*)-**23** (79%), respectively, as white foams. Compound (*R*)-**23**: $^{1}$H NMR (400 MHz, $CDCl_3$) 1.30 and 1.32 (2 × t, $^{3}J_{H,H}$ = 7.1 Hz, 2 × 3H), 3.52 (dd, $^{3}J_{H,H}$ = 6.1 Hz, $^{2}J_{H,H}$ = 10.2 Hz, 1H), 3.57 (dd, $^{3}J_{H,H}$ = 4.4 Hz, $^{2}J_{H,H}$ = 10.2 Hz, 1H), 4.06 – 4.30 (m, 5H), 4.44 (dd, $^{3}J_{H,H}$ = 4.8 Hz, $^{2}J_{H,H}$ = 14.2 Hz, 1H), 5.05 – 5.14 (m, 1H), 5.42 (d, $^{3}J_{H,H}$ = 7.9 Hz, 1H), 6.91 (d, $^{3}J_{H,H}$ = 7.9 Hz, 1H), 7.12 – 7.32 (m, 15H), 8.21 (s, 1H), 9.46 (br s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) 16.4 (d, $^{3}J_{C,P}$ = 6.4 Hz), 49.7, 59.8, 63.3 and 63.4 (2 × d, $^{2}J_{C,P}$ = 5.6 Hz), 63.5,

87.7, 102.4, 127.3, 127.6, 128.0, 128.1, 128.3, 128.4, 132.3 (d, $^{2}J_{C,P}$ = 33.0 Hz), 137.8 (d, $^{1}J_{C,P}$ = 238.7 Hz), 142.8, 144.7, 150.9, 163.4; $^{31}$P NMR (161.9 MHz, $CDCl_3$) 6.86; HRMS *m/z* calcd for $C_{32}H_{34}N_5NaO_6P$ $[M+Na]^+$ 638.21389, found 638.21391. $[\alpha]^{20}_D$ +59.7 (*c* 0.67, MeOH). Compound (*S*)-**23**: $^{1}$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**23**; HRMS *m/z* calcd for $C_{32}H_{34}N_5NaO_6P$ $[M+Na]^+$ 638.21389, found 638.21390. $[\alpha]^{20}_D$ –57.3 (*c* 0.44, MeOH).

**Diethyl (*R*)-1-[1-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*R*)-24 and Diethyl (*S*)-1-[1-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*S*)-24.** Treatment of (*R*)-**23** or (*S*)-**23** by *GP4*, followed by flash chromatography over silica gel ($CHCl_3$/MeOH, 95/5 → $CHCl_3$/MeOH, 90/10) afforded (*R*)-**24** (80%) and (*S*)-**24** (67%), respectively, as white hygroscopic foams. Compound (*R*)-**24**: $^{1}$H NMR (400 MHz, DMSO-*d6*) 1.22 and 1.23 (2 × t, $^{3}J_{H,H}$ = 7.0 Hz, 2 × 3H), 3.82 – 4.09 (m, 6H), 4.14 (dd, $^{3}J_{H,H}$ = 9.6 Hz, $^{2}J_{H,H}$ = 14.3 Hz, 1H), 4.21 (dd, $^{3}J_{H,H}$ = 4.9 Hz, $^{2}J_{H,H}$ = 14.3 Hz, 1H), 5.02 – 5.12 (m, 1H), 5.39 (d, $^{3}J_{H,H}$ = 7.9 Hz, 1H), 7.26 (d, $^{3}J_{H,H}$ = 7.9 Hz, 1H), 8.74 (s, 1H), 11.27 (br s, 1H); $^{13}$C NMR (100 MHz, DMSO-*d6*) 16.0 (d, $^{3}J_{C,P}$ = 6.2 Hz), 48.4, 60.5, 61.3, 62.2 and 62.3 (2 × d, $^{2}J_{C,P}$ = 5.4 Hz), 101.0, 131.2 (d, $^{2}J_{C,P}$ = 33.6 Hz), 136.2 (d, $^{1}J_{C,P}$ = 236.4 Hz), 145.0, 150.7, 163.4; $^{31}$P NMR (161.9 MHz, DMSO-*d6*) 8.14; HRMS *m/z* calcd for $C_{13}H_{20}N_5NaO_6P$ $[M+Na]^+$ 396.10434, found 396.10429. $[\alpha]^{20}_D$ +156.5 (*c* 0.41, MeOH). Compound (*S*)-**24**: $^{1}$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**24**; HRMS *m/z* calcd for $C_{13}H_{20}N_5NaO_6P$ $[M+Na]^+$ 396.10434, found 396.10425. $[\alpha]^{20}_D$ –148.5 (*c* 0.34, MeOH).

**(*R*)-1-[1-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonic acid (*R*)-25 and (*S*)-1-[1-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonic acid (*S*)-25.** Treatment of (*R*)-**24** or (*S*)-**24** by *GP2* afforded, after crystallization from a water-MeOH (1:5) mixture, (*R*)-**25** (91%) and (*S*)-**25** (98%), respectively, as white crystals. Compound (*R*)-**25**: decomposition at 260 °C; $^{1}$H NMR (400 MHz, DMSO-*d6*) 3.78 – 3.87 (m, 2H), 4.14 (dd, $^{3}J_{H,H}$ = 9.5 Hz, $^{2}J_{H,H}$ = 14.3 Hz, 1H), 4.23 (dd, $^{3}J_{H,H}$ = 4.9 Hz, $^{2}J_{H,H}$ = 14.3 Hz, 1H), 4.99 – 5.09 (m, 1H), 5.40 (d, $^{3}J_{H,H}$ = 7.9 Hz, 1H), 7.22 (d, $^{3}J_{H,H}$ = 7.9 Hz, 1H), 8.44 (s, 1H), 11.31 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-*d6*) 48.6, 60.8, 60.9, 101.0, 128.9 (d, $^{2}J_{C,P}$ = 32.3 Hz), 141.4 (d, $^{1}J_{C,P}$ = 229.7 Hz), 145.1, 150.8, 163.5; $^{31}$P NMR (161.9 MHz, DMSO-*d6*) 3.26; HRMS *m/z* calcd for $C_9H_{11}N_5O_6P$ $[M-H]^-$ 316.04524, found 316.04542. $[\alpha]^{20}_D$ +169.9 (*c* 0.35, $H_2O$). Compound

(*S*)-**25**: decomposition at 262 °C; $^{1}$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**25**; HRMS *m/z* calcd for $C_9H_{11}N_5O_6P$ $[M-H]^-$ 316.04524, found 316.04554. $[\alpha]^{20}_D$ –173.2 (*c* 0.37, $H_2O$).

**Diethyl (*R*)-1-[1-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-(trityloxy)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*R*)-26 and Diethyl (*S*)-1-[1-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-(trityloxy)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*S*)-26.** Treatment of (*R*)-**12** or (*S*)-**12** with $N^3$-benzoylthymine by *GP3*, followed by column chromatography over silica gel (hexan/$CHCl_3$/MeOH, 6/4/0.1 → hexan/$CHCl_3$/MeOH, 6/4/0.4), afforded crude $N^3$-benzoylthymine intermediates. Propylamine (3 mL) was added to the solutions of the crude intermediates in dioxane (30 mL) and the reaction mixture was stirred at room temperature for 12 h. Column chromatography over silica gel (hexane/$CHCl_3$/MeOH, 6/4/0.2) afforded (*R*)-**26** (64%) and (*S*)-**26** (65%), respectively, as white foams. Compound (*R*)-**26**: $^{1}$H NMR (400 MHz, $CDCl_3$) 1.30 and 1.32 (2 × t, $^{3}J_{H,H}$ = 7.1 Hz, 2 × 3H), 1.71 (s, 3H), 3.51 – 3.61 (m, 2H), 4.08 – 4.27 (m, 5H), 4.40 (dd, $^{3}J_{H,H}$ = 4.7 Hz, $^{2}J_{H,H}$ = 14.2 Hz, 1H), 5.05 – 5.15 (m, 1H), 6.72 (s, 1H), 7.18 – 7.35 (m, 15H), 8.18 (s, 1H), 9.17 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) 12.2, 16.4 (d, $^{3}J_{C,P}$ = 6.5 Hz), 49.7, 60.0, 63.2 and 63.3 (2 × d, $^{2}J_{C,P}$ = 5.6 Hz), 63.6, 87.7, 111.1, 127.4, 127.6, 128.0, 128.1, 128.3, 128.5, 132.1 (d, $^{2}J_{C,P}$ = 33.0 Hz), 137.9 (d, $^{1}J_{C,P}$ = 238.8 Hz), 140.4, 142.9, 150.9, 163.8; $^{31}$P NMR (161.9 MHz, $CDCl_3$) 6.98; HRMS *m/z* calcd for $C_{33}H_{36}N_5NaO_6P$ $[M+Na]^+$ 652.22954, found 652.22952. $[\alpha]^{20}_D$ +54.7 (*c* 0.49, MeOH). Compound (*S*)-**26**: $^{1}$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**26**; HRMS *m/z* calcd for $C_{33}H_{36}N_5NaO_6P$ $[M+Na]^+$ 652.22954, found 652.22951. $[\alpha]^{20}_D$ –47.2 (*c* 0.50, MeOH).

**Diethyl (*R*)-1-[1-hydroxy-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*R*)-27 and Diethyl (*S*)-1-[1-hydroxy-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*S*)-27.** Treatment of (*R*)-**26** or (*S*)-**26** by *GP4*, followed by flash chromatography over silica gel ($CHCl_3$/MeOH, 95/5 → $CHCl_3$/MeOH, 90/10) afforded (*R*)-**27** (82%) and (*S*)-**27** (84%), respectively, as white hygroscopic foams. Compound (*R*)-**27**: $^{1}$H NMR (400 MHz, DMSO-*d6*) 1.22 and 1.23 (2 × t, $^{3}J_{H,H}$ = 7.0 Hz, 2 × 3H), 1.60 (s, 3H), 3.82 – 4.08 (m, 6H), 4.10 (dd, $^{3}J_{H,H}$ = 9.7 Hz, $^{2}J_{H,H}$ = 14.3 Hz, 1H), 4.17 (dd, $^{3}J_{H,H}$ = 4.8 Hz, $^{2}J_{H,H}$ = 14.3 Hz, 1H), 5.02 – 5.12 (m, 1H), 5.35 (t, $^{3}J_{H,H}$ = 5.4 Hz, 1H), 7.14 (s, 1H), 8.73 (s, 1H), 11.26 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-*d6*) 11.7, 16.0 (d, $^{3}J_{C,P}$ = 6.2 Hz), 48.2, 60.5, 61.4, 62.2 and 62.3 (2 × d, $^{2}J_{C,P}$ =

5.9 Hz), 108.5, 131.2 (d, $^{2}J_{C,P}$ = 33.7 Hz), 136.2 (d, $^{1}J_{C,P}$ = 236.6 Hz), 140.7, 150.7, 164.0; $^{31}$P NMR (161.9 MHz, DMSO-*d6*) 8.17; HRMS *m/z* calcd for $C_{14}H_{22}N_5NaO_6P$ $[M+Na]^+$ 410.11999, found 410.11997. $[\alpha]^{20}_D$ +131.0 (*c* 0.29, MeOH). Compound (*S*)-**27**: $^{1}$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**27**; HRMS *m/z* calcd for $C_{14}H_{22}N_5NaO_6P$ $[M+Na]^+$ 410.11999, found 410.12012. $[\alpha]^{20}_D$ –136.3 (*c* 0.57, MeOH).

**(*R*)-1-[1-Hydroxy-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonic acid (*R*)-28 and (*S*)-1-[1-Hydroxy-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonic acid (*S*)-28.** Treatment of (*R*)-**27** or (*S*)-**27** by *GP2* afforded, after crystallization from a water-EtOH-EtOAc-acetone (1:5:30:30) mixture, (*R*)-**28** (88%) and (*S*)-**28** (91%), respectively, as white crystals. Compound (*R*)-**28**: decomposition at 250 °C; $^{1}$H NMR (400 MHz, DMSO-*d6*) 1.62 (s, 3H), 3.78 – 3.89 (m, 2H), 4.09 (dd, $^{3}J_{H,H}$ = 9.4 Hz, $^{2}J_{H,H}$ = 14.3 Hz, 1H), 4.18 (dd, $^{3}J_{H,H}$ = 5.0 Hz, $^{2}J_{H,H}$ = 14.3 Hz, 1H), 4.99 – 5.09 (m, 1H), 7.10 (s, 1H), 8.43 (s, 1H), 11.28 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-*d6*) 11.8, 48.2, 60.8, 60.9, 108.5, 128.9 (d, $^{2}J_{C,P}$ = 32.4 Hz), 140.8, 141.4 (d, $^{1}J_{C,P}$ = 229.9 Hz), 150.8, 164.0; $^{31}$P NMR (161.9 MHz, DMSO-*d6*) 3.62; HRMS *m/z* calcd for $C_{10}H_{13}N_5O_6P$ $[M-H]^-$ 330.06089, found 330.06086. $[\alpha]^{20}_D$ +151.1 (*c* 0.32, $H_2O$). Compound (*S*)-**28**: decomposition at 238 °C; $^{1}$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**28**; HRMS *m/z* calcd for $C_{10}H_{13}N_5O_6P$ $[M-H]^-$ 330.06089, found 330.06120. $[\alpha]^{20}_D$ –149.1 (*c* 0.46, $H_2O$).

**Diethyl (*R*)-1-[1-(4-amino-2-oxopyrimidin-1(2*H*)-yl-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*R*)-29 and Diethyl (*S*)-1-[1-(4-amino-2-oxopyrimidin-1(2*H*)-yl-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonate (*S*)-29.**

Treatment of (*R*)-**12** or (*S*)-**12** with $N^4$-benzoylcytosine by *GP3*, followed by column chromatography over silica gel, afforded crude $N^4$-benzoylcytosine intermediates. Propylamine (3 mL) was added to the solutions of the crude intermediates in dioxane (30 mL) and the reaction mixture was stirred at room temperature for 12 h. Column chromatography over silica gel (hexane/$CHCl_3$/MeOH, 6/4/0.1 → hexane/$CHCl_3$/MeOH, 6/4/0.7) afforded debenzoylated cytosine intermediates as white foams. Treatment of the cytosine intermediates by *GP4*, followed by flash chromatography over silica gel ($CHCl_3$/MeOH, 95/5 → $CHCl_3$/MeOH, 7/3) afforded (*R*)-**29** (40%) and (*S*)-**29** (38%), respectively, as white hygroscopic foams. Compound (*R*)-**29**: $^{1}$H NMR (400 MHz, DMSO-*d6*) 1.22 and 1.23 (2 × t, $^{3}J_{H,H}$ = 7.0 Hz, 2 × 3H), 3.78 – 3.91 (m, 2H), 3.94 – 4.10 (m, 5H), 4.24 (dd, $^{3}J_{H,H}$ = 4.6 Hz,

$^{2}J_{H,H}$ = 13.9 Hz, 1H), 5.09 – 5.18 (m, 1H), 5.43 (d, $^{3}J_{H,H}$ = 7.2 Hz, 1H), 6.99 (br s, 1H), 7.07 (br s, 1H), 7.12 (d, $^{3}J_{H,H}$ = 7.2 Hz, 1H), 8.69 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-*d6*) 16.0 (d, $^{3}J_{C,P}$ = 6.2 Hz), 49.8, 60.9, 61.4, 62.2 and 62.3 (2 × d, $^{2}J_{C,P}$ = 5.5 Hz), 93.3, 131.2 (d, $^{2}J_{C,P}$ = 33.4 Hz), 136.1 (d, $^{1}J_{C,P}$ = 236.6 Hz), 145.4, 155.5, 165.9; $^{31}$P NMR (161.9 MHz, DMSO-*d6*) 8.49; HRMS *m/z* calcd for $C_{13}H_{21}N_6NaO_5P$ $[M+Na]^+$ 395.12033, found 395.12024. $[\alpha]^{20}_D$ +182.5 (*c* 0.32, MeOH). Compound (*S*)-**29**: $^{1}$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**29**; HRMS *m/z* calcd for $C_{13}H_{21}N_6NaO_5P$ $[M+Na]^+$ 395.12033, found 395.12031. $[\alpha]^{20}_D$ – 172.1 (*c* 0.41, MeOH).

**(*R*)-1-[1-(4-Amino-2-oxopyrimidin-1(2*H*)-yl-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonic acid (*R*)-30 and (*S*)-1-[1-(4-Amino-2-oxopyrimidin-1(2*H*)-yl-3-hydroxypropan-2-yl]-1*H*-1,2,3-triazol-4-ylphosphonic acid (*S*)-30.** Treatment of (*R*)-**29** or (*S*)-**29** by *GP2* afforded, after crystallization from a water-EtOH (1:1) mixture, (*R*)-**30** (71%) and (*S*)-**30** (87%), respectively, as white crystals. Compound (*R*)-**30**: decomposition at 265 °C; $^{1}$H NMR (400 MHz, DMSO-*d6*) 3.82 (d, $^{3}J_{H,H}$ = 5.2 Hz, 2H), 4.07 – 4.21 (m, 1H), 4.23 – 4.35 (m, 1H), 5.01 – 5.13 (m, 1H), 5.66 (d, $^{3}J_{H,H}$ = 7.2 Hz, 1H), 7.33 (d, $^{3}J_{H,H}$ = 7.2 Hz, 1H), 8.15 (br s, 2H), 8.38 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-*d6*) 49.8, 60.6, 61.0, 93.5, 128.5 (d, $^{2}J_{C,P}$ = 31.8 Hz), 142.3 (d, $^{1}J_{C,P}$ = 226.5 Hz), 147.1, 152.5, 163.3; $^{31}$P NMR (161.9 MHz, DMSO-*d6*) 2.94; HRMS *m/z* calcd for $C_9H_{12}N_6O_5P$ $[M-H]^-$ 315.06123, found 315.06119. $[\alpha]^{20}_D$ +204.4 (*c* 0.45, $H_2O$). Compound (*S*)-**30**: decomposition at 265 °C; $^{1}$H, $^{13}$C and $^{31}$P NMR spectra are identical with (*R*)-**30**; HRMS *m/z* calcd for $C_9H_{12}N_6O_5P$ $[M-H]^-$ 315.06123, found 315.06125. $[\alpha]^{20}_D$ –198.2 (*c* 0.53, $H_2O$).

**Determination of $K_i$ values for human HGPRT, *Pf*HGXPRT and *Pv*HGPRT.[8]**

Human HGPRT and *Pv*HGPRT were stored in 0.1 M Tris-HCl, 0.01 M $MgCl_2$, pH 7.4, 200 μM *P*Rib-*PP*, 1 mM dithiothreitol (DTT), at −80 °C, while *Pf*HGXPRT in 0.01 M phosphate, 60 μM hypoxanthine, 200 μM *P*Rib-*PP*, at pH 7.2, 1 mM DTT as previously described.[29] The buffer was 0.1 M Tris-HCl, 0.01 M $MgCl_2$, pH 7.4 for the enzyme assays. For the *Pf* and *Pv* enzymes, the assays were performed in this buffer and also in 0.01 M phosphate, 5 mM DTT as described by Hazelton and colleagues.[30] The $K_i$ values were calculated by Hanes' plots at a fixed concentration of guanine (60 μM) and at variable concentrations of *P*Rib-*PP* (14−1000 μM) depending on the *K*m(app) in the presence of the inhibitor.

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